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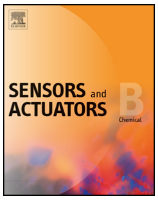

Benzimidazole functionalised Schiff bases: Novel pH sensitive fluorescence turn-on chromoionophores for ion-selective optodes

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ABSTRACT

Benzimidazole-based Schiff bases 1–3, designed as D-π-A chromophores, are presented as novel pH responsive chromo- and fluoroionophores for ion-selective optodes. Tuning of their ICT character and photophysical properties has been achieved by introduction of an electron donating *N*,*N*-diethyl amino group and strong electron withdrawing groups $-$CN and $-NO_2$ on the benzimidazole acceptor moiety. The core molecular structure also contains a salicylidene moiety that potentially introduces photoinduced proton transfer reactivity. In general, pH chemosensors based on Schiff bases are relatively rare due to the common problem of protonation induced hydrolysis of the imino bond in water. The photophysical and sensing properties of 1–3 are characterised in solutions and thin polymer films, and the corresponding apparent pK_a and pK_a^* values are determined. In ethanol/water solutions, Schiff bases 1–3 show very weak fluorescence with reversible naked-eye visible colour change upon protonation (apparent $pK_a \sim 4$). Upon immobilisation in plasticised PVC films the photophysical properties and pH responses of 1–3 significantly change resulting in reversible fluorimetric turn-on and turn-off pH sensitivity with apparent pK_a values in the physiologically relevant pH range (apparent $pK_a \sim 6$-7). A reversible spectroscopic response to pH is achieved because protonation of the immobilised benzimidazole Schiff bases occurs on the stable benzimidazole moiety (electron acceptor), whilst the imino bond remains preserved. An application for these novel fluoroionophores is demonstrated with a fluorescence turn-on potassium selective optode, based on an ion-exchange sensing mechanism.

1. Introduction

Electron donor-π-electron acceptor molecular systems (D-π-A systems) based on charge-transfer interactions are suitable for design of optical chemosensors due to their excellent properties as chromophores and fluorophores, as well as their varied ability to interact with potential analytes. Schiff bases play an important role in such systems, and represent one of the most investigated classes of organic compounds [1]. Besides their broad spectrum of biological activity [2,3], they are often used as catalysts and intermediates in organic synthesis [4], as corrosion inhibitors [5,6], as chelating agents [7,8] and consequently in optical chemical sensors for detection of metal ions [9,10] and as chemodosimeters [11].

pH responsive Schiff bases are often used as fluorescent probes for imaging [12–18], as colorimetric indicators [19], and as fluorescent chemosensors based on aggregation induced emission (AIE) [20,21]. However, the use of Schiff bases for pH sensing in an aqueous environment is often problematic due to complex protonation and tautomeric equilibria involving a number of different species, and due to hydrolysis of the imino bond in polar solvents [22]. Hence, development of reversible pH sensors based on Schiff base derivatives is challenging and requires careful consideration of their structure-property relationships.

The benzimidazole moiety is another attractive building block of D-π-A molecular structures due to the multifunctionality of its heteroaromatic conjugated planar structure. The photophysical properties of benzimidazole based compounds can significantly change upon protonation/deprotonation [23–26], during metal-ion chelating and in interaction with biomolecules [27–29]. The combination of benzimidazole and Schiff base building blocks in a single molecular entity have been successfully used for optical detection of various metal ions [30–34], and the fluoride anion [35] in solutions.

One way of improving the stability of Schiff bases and preserving their pH sensitivity is by immobilisation in an appropriate material, as has been demonstrated with pH test strips based on organometal

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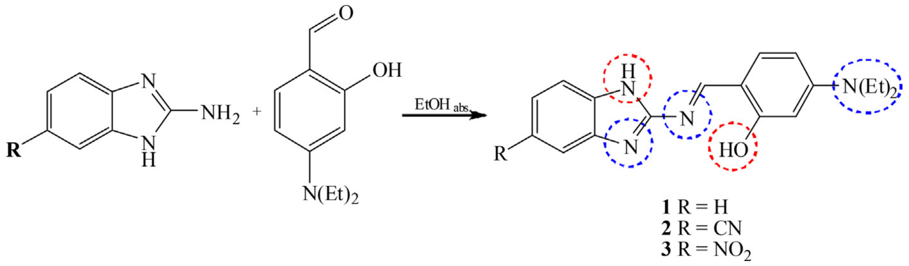

Scheme 1. Chemosensors 1-3 [3]. Protonable (blue) and deprotonable (red) sites are specified. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

complexes [12,36], fiber optic pH sensors [37,38] and pH sensitive optodes [39]. In optodes, Schiff bases are typically incorporated into PVC thin films as metal ion responsive chromophores or fluorophores [40–44], and less often as pH sensitive fluoroionophores [37].

In this work as part of our ongoing research effort on developing benzimidazole based ICT chromophores for pH and metal-ion sensing [23,24,29], we have designed and characterised benzimidazole based Schiff bases 1–3 (Scheme 1) for the reversible fluorimetric determination of pH and for potential use in ion-selective optodes. The multifunctional pH responsive benzimidazole moiety offers alternative imino nitrogen as a protonation site and contributes to the photophysical and sensing properties of 1–3. Immobilisation of 1–3 in lipophilic PVC matrices prevents hydrolysis of the Schiff bases, shifts the pK_a values and improves their fluorescence intensity as a result of the change of molecular microenvironment. The function of synthesised benzimidazole based Schiff bases as fluoroionophores is demonstrated in an ion-selective optode for potassium ion determination.

2. Experimental

2.1. Materials and instrumentation

All chemicals and solvents for synthesis and characterisation were purchased from commercial suppliers Acros, Aldrich or Fluka. Methanol, hydrochloride acid, sodium hydroxide and organic solvents were obtained from Kemika d.d., Zagreb. High molecular weight poly(vinylchloride) (PVC), bis(2-ethylhexyl) sebacate (DOS), cation-exchanger potassium tetrakis(4-chlorophenyl) borate, bis(2-ethylhexyl) sebacate (DOS), Potassium ionophore I (valinomycin), and tetrahydrofuran (THF) were purchased from Fluka (Switzerland). Milli-Q water was used for the preparation of aqueous solutions. pH in the range 1–13 was measured on commercially available combination pH electrode BlueLine 17 pH (Schott AG, Mainz, Germany). Absorbance spectra were recorded on a Carry 100 Scan Varian spectrophotometer. Fluorescence measurements were carried out on a Varian Cary Eclipse fluorescence spectrophotometer.

2.2. Synthesis and spectral characterisation of 1–3

All compounds presented in Fig. 1 were prepared by method previously reported by our research group [3] and characterised by ^{1}H NMR and ^{13}C NMR spectroscopy. Schiff bases were prepared from 2-aminobenzimidazoles and corresponding aromatic aldehydes in absolute ethanol for 12–24 h at reflux. Detailed description can be found in Electronic supporting information materials.

The stock solutions for **1**-**3** were prepared in ethanol (10 mM). Spectrophotometric titrations were carried out using 1×10^{-5} mol dm^{-3} solutions of the compounds **1**-**3** in ethanol or buffer solutions for the absorbance measurements and 2×10^{-6} mol dm^{-3} solutions for the fluorescence measurements. Absorbance measurements were carried out using quartz cells of 1 cm path length and absorbance values were recorded at 1 nm. Wavelength scan was performed between 200 nm and 800 nm. Baseline was recorded prior to each set of experiments. Fluorescence measurements were carried out on a Varian Cary Eclipse fluorescence spectrophotometer at 25 °C using 1 cm path quartz cells. Excitation wavelengths were determined from absorbance maxima. Emission spectra were recorded from 300 nm to 800 nm and corrected for the effects of time and wavelength dependent light source fluctuations using a standard of Rhodamine 101, a diffuser provided with the fluorimeter and the software supplied with the instrument. Relative fluorescence quantum yields were determined according to *Miller* using Eq. (1):

$$\Phi_x = \Phi_s \times A_s D_x {n_x}^2 / A_x D_s {n_s}^2 \quad (1)$$

where in Φ is the emission quantum yield, A is the absorbance at the excitation wavelength, D is the area under the corrected emission curve and n is the refractive index of the solvents used. The subscripts s and x refer to the standard and to the unknown, respectively. The standard employed was quinine sulphate with a published fluorescence quantum yield of 0.54 [45]. All samples were purged with nitrogen to displace oxygen. pH adjustment of ethanol solutions (EtOH:H$_2$O 95:5 v/v) was made with concentrated hydrochloride acid (HCl) and sodium hydroxide (NaOH). Apparent pK_a values were determined spectrophotometrically from fitted data points using the Boltzmann function.

Due to low solubility, measurements were conducted by diluting stock solutions of **1-3** in ethanol (volume fraction of ethanol in measured solutions is less than 1%).

2.3. Immobilisation and pH response of 1–3 in thin polymer films

Chromophores 1–3 were immobilised in plasticised PVC matrix starting from liquid mixture prepared by modified method found in literature [46]. The 'cocktail' contained 67 mg PVC, 134 mg DOS (the plasticiser), 2.4 mg (1 eq) of PTCB and 1 eq of compound 1–3 in 1.5 mL THF. Mixture was placed in ultrasonic bath for 15 min. Thin polymer films were prepared by spreading 100 μL mixture onto a 2.5 × 2.5 cm solid transparent polyester sheet by spin-coating technique. Obtained thin films were dried at room temperature for 18 h in dark. pH response of immobilised chromophores was examined in buffer solutions pH 2–12. Apparent pK_a values were determined spectrophotometrically from fitted data points using the Boltzmann function.

2.4. Preparation and characterisation of polymeric optodes

'Cocktails' for preparation of ion-selective optodes were prepared by diluting PVC in dry THF. The cocktail contained 67 mg PVC, 134 mg DOS (the plasticiser), 3.1 mg of PTCB, 7.1 mg of valinomycin and 0.9 mg of compound **3** in 1.5 mL THF. The mixture was placed